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Evaluation of the i-STAT portable point-of-care analyzer for determination of blood gases and acid-base status in newborn calves

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Abstract: **OBJECTIVE:** To validate the i-STAT, a portable hand-held analyzer that allows point-of-care measurement of blood gases, acid-base, lactate, and other blood variables in food animal practice for analysis of blood samples of newborn calves. **DESIGN:** Prospective observational study. **SETTING:** University teaching hospital. **MEASUREMENTS AND MAIN RESULTS:** A total of 271 venous blood samples were analyzed for PvO₂, PvCO₂, pH, base excess (BE), HCO₃, venous saturation of oxygen (SvO₂), and total carbon dioxide (TCO₂) using an i-STAT and a Rapidlab 248 bench top analyzer that served as the reference method. Lactate was measured using the i-STAT as well as photometrically. Results from the i-STAT and the reference methods were compared. The analytes BE, HCO₃, and TCO₂ showed a constant systematic error across the entire range with 2.3, 1.9, and 2.0 mmol/L lower values, respectively, than the values measured by the reference method. Based on the combined inherent imprecision of the 2 analyzers and after correcting the influences of systematic errors, the PvO₂, HCO₃, and SvO₂ were within the acceptable limits in 76% to 91% of the cases. Ninety-five percent of the measurements of PvCO₂ and BE were within acceptable limits. **CONCLUSIONS:** The overall performance of the i-STAT was good except for BE, HCO₃, and TCO₂, thus limiting its usefulness in clinical studies. However this hand-held device allows rapid, reliable, and accurate point-of-care blood analyses and thus can be useful in bovine practice.

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Abbreviations

sO₂ oxygen saturation

BE base excess

TCO₂ total carbon dioxide

Abstract

Objective – The purpose of this study was to validate the i-STAT, a portable handheld analyzer that allows point-of-care measurement of blood gases, acid-base, lactate and other blood variables in food animal practice for analysis of blood samples of newborn calves.

Design – Prospective study.

Setting – University teaching hospital.

Measurements and Main Results – A total of 271 venous blood samples were analyzed for PO₂, PCO₂, pH, BE, HCO₃, sO₂, and TCO₂ using an i-STAT and a Rapidlab 248 bench top analyzer that served as the reference method. L-lactate was measured using the i-STAT as well as photometrically. Results from the i-STAT and the reference methods were compared. BE, HCO₃ and TCO₂ showed a constant systematic error across the entire range with 2.3 mmol/L, 1.9 mmol/L and 2.0 mmol/L lower values than the values from the reference method. Based on the combined inherent imprecision of the two analyzers and after correcting the influences of systematic errors the PO₂, HCO₃, and sO₂ were within the acceptable limits in 76 to 91% of the cases. 95% of the measurements of PCO₂ and BE were in within the limits.

Conclusions – The overall performance of the i-STAT was good with the mentioned limitations, thus limiting its usability in clinical studies. However this handheld device allows rapid, reliable, and accurate point-of-care blood analyses and thus improves diagnostics in bovine practice.

Keywords: acid-base status, asphyxia, blood gas, calves, i-STAT

24

25 ***Introduction***

26 Perinatal calf mortality is an increasing problem globally.¹ The majority of perinatal deaths occur during
27 or within 24 hours of birth and the most common cause is asphyxia during parturition.² In addition to
28 hypoxia, calves suffer from hypercapnia of arterial and venous blood and mixed respiratory and metabolic
29 acidosis.³ Resolution of this type of acidosis is critical for the survival of calves.⁴ Until recently, accurate
30 determination of acid-base status could only be achieved in a hospital setting, and the severity of acidosis
31 in the field was therefore estimated using various clinical variables.⁵⁻⁷ However, the introduction of
32 handheld devices such as the i-STAT analyzer^a has allowed patient-side analysis of certain variables in a
33 variety of animal species including humans.⁸⁻¹¹ The i-STAT has been used to evaluate blood samples from
34 a limited number of adult healthy cattle, but not calves with acidosis.¹² The goal of the present study was
35 to analyze blood gases, acid-base status and L-lactate concentration in venous blood using the i-STAT in
36 newborn calves with mixed respiratory-metabolic acidosis and compare the results with those from a
37 conventional blood-gas analyzer.

38

39

40 ***Materials and Methods***

41 **Blood samples**

42 Forty-six calves delivered by Caesarian section (n = 28) or naturally using light manual traction (n = 18) at
43 the teaching hospital, were used in the study. The births occurred over a 20-month period. The 46 calves
44 comprised 22 Swiss Braunvieh, 8 Holstein Friesian, 9 Red Holstein, 5 Simmental and 2 Limousin calves,
45 and there was one set of twins. Thirty calves were male and 16 female and the average body weight of the
46 calves was 45.5 ± 6.3 kg. The dams had been referred from private practice because of dystocia, were

patients in the clinic at the time of calving, or belonged to the university and were shipped to the hospital for calving. Based on the initial venous blood pH measured with the reference method, acidemic calves ($n = 15$) with a blood pH < 7.2 were treated with intravenous administration of sodium bicarbonate solution (600 mmol $\text{Na}^+\text{HCO}_3^-$ per 1000 mL) within 15 minutes of birth.¹³ The amount was calculated using the formula:

$$\text{mmol sodium bicarbonate} = (-\text{base excess}) \times 0.5 \times \text{kg body weight.}$$

Blood samples were collected from the left or right jugular vein immediately after birth using a needle^b (1.2 x 40 mm). Subsequent samples were collected through an indwelling 1.8 x 16 mm jugular venous catheter,^c which was placed after shaving and cleaning the skin with alcohol. The catheter was secured to the skin with non-absorbable suture material.^d Before blood collection, 3 mL of blood was removed from the catheter using a syringe. Each blood sample was collected into a blood gas syringe that contained calcium-balanced lithium heparin in a concentration of 50 IU/mL blood.^e Gas bubbles were removed and the blood stored at room temperature and analyzed with both analyzers within 15 minutes of collection. After blood collection, the catheter was rinsed with 3 mL isotonic NaCl solution^f with 10 IU/mL added heparin.^g Blood samples for determination of L-lactate concentration were collected into vacuum tubes containing sodium fluoride^h and centrifuged, and the harvested plasma was stored at -20°C for further analysis. In 16 patients, blood was collected 0, 20, 40 minutes and 1, 2, 4, 12, 24, 48 and 72 hours post natum. In the remaining 30 calves, blood was collected at birth and 20, 40 and 60 minutes post natum. The only time L-lactate was not determined was 20 minutes post natum. Because 9 samples were not collected, there were 271 samples evaluated with the i-STAT and the reference blood gas machine, and 225 samples were evaluated for L-lactate with the reference method.

Hand-held analyzer

The i-STAT is a hand-held, portable, battery-powered point-of-care analyzer. It has an integrated thermometer, barometer and memory for the last 50 measurements, which are displayed on a screen and can be uploaded to a printer. The analyzer consists of a handheld machine and single-use cartridges that

combine various miniaturized microsensors, a single-point calibration system, fluid channel, and waste chamber. The type CG4+ cartridge used in the present study allowed the measurement of pH, PCO₂, PO₂, and the concentration of L-lactate (mmol/L). From these results, the following variables were calculated: oxygen saturation (sO₂; %), HCO₃, base excess (BE; mmol/L), and total carbon dioxide (TCO₂; mmol/L). The cartridge includes biosensor chips for determination of direct ion-selective potentiometry (pH, PCO₂), amperometry (PO₂), and enzyme-based amperometry (L-lactate). Approximately 60 µl of blood was placed in a preformed sample entry well in the cartridge, which was then inserted in the analyzer. The analyzer started automatically after insertion of the cartridge, ran a test program, underwent self-calibration, warmed the sensors to 37°C, and then analyzed the blood sample. Test results were displayed on the screen 120 seconds later.

Blood pH was measured by direct potentiometry and the reportable range was 6.50 to 8.20.¹⁴ For calculation of pH, the H⁺ ion concentration is related to the H⁺ potential of blood through the Nernst equation. PCO₂ is also measured via direct ion-selective potentiometry within a range of 5 to 130 mmHg and related to the CO₂-potential of blood through the same equation. Partial pressure of oxygen is measured amperometrically within a range of 5 to 800 mmHg using Clarke-type electrodes. L-lactate concentration is measured amperometrically with a reporting range of 0.30 to 20.00 mmol/L. The enzyme lactate oxidase that is immobilized in the biosensor selectively converts lactate to pyruvate and hydrogen peroxide. The latter is oxidized at a platinum electrode and the electrical current produced is proportional to the lactate concentration of the sample. Bicarbonate is indicated within a range of 1.0 to 85.0 mmol/L and the actual bicarbonate concentration (HCO_{3act}) is calculated using the formula:

$$\log \text{HCO}_{3\text{act}} = \text{pH} + \log \text{PCO}_2 - 7.608$$

BE of the extracellular fluid (BE_{ecf}) is indicated within a range of ± 30.00 mmol/L and its concentration is calculated using the formula:

$$\text{BE}_{\text{ecf}} = \text{cHCO}_{3\text{act}} - 24.8 + 16.2 * (\text{pH} - 7.4).$$

Oxygen saturation is a relative value and is expressed as the percentage of the amount of transported O₂ relative to the maximum possible amount of transported O₂. It is indicated within a range of 1% to 100% and calculated using the formula:

$$sO_2 = \frac{100 * (X^3 + 150 * X)}{X^3 + 150 * X + 23400}$$

In which $X = PO_2 * 10^{((0.48 * (pH - 7.4) - 0.0013 * (HCO_3 - 25)))}$.

i-STAT calculates TCO₂ from pH and PCO₂ using the standardized Henderson-Hasselbalch equation:

TCO₂ = HCO₃ + 0.03*PCO₂. The reporting range for TCO₂ is 5 to 50 mmol/L.

Reference methods

Concurrent measurements were conducted of all samples using the Rapidlab 248 blood gas analyzer.¹ For direct comparison of blood gas values, the default temperature of analysis of the Rapidlab 248 of 37°C was not adjusted to the body temperature of the patients because the temperature setting of 37°C cannot be changed in the i-STAT.

Blood gas analyzer

The Rapidlab 248 is a bench top blood-gas analyzer, which uses sensor electrode technique to measure pH, PCO₂, PO₂, and the variables calculated by the i-STAT. The test sample is introduced into the analyzer semiautomatically via an aspiration needle. After an analysis time of 45 seconds the results are displayed on a screen as well as on a print out. Quality controls were conducted twice weekly with at least 2 of the 3 levels of control.

Blood pH is measured within a range of 6.00 – 8.00, which corresponds to a proton concentration of 10.0 – 997.7 nmol/L. The pH sensor uses ion-selective electrodes and forms the half-cell that supplements the external reference sensor. The difference in potential between the two sensors reflects the

proton concentration of the test sample and is used for calculation of the pH. The PCO_2 is proportional to the quotient of dissolved CO_2/HCO_3 and is measured within a range of 5.0 to 250 mmHg. The PCO_2 sensor consists of a measuring electrode and an internal reference electrode, and the difference in potential of the two electrodes corresponds to a change in pH at the reference sensor. The change in pH is proportional to the logarithm of PCO_2 . PO_2 is measured amperometrically using Clarke-type electrodes within a range of 0.0 to 749.0 mmHg. Calculation of BE is the same as with i-STAT. The Rapidlab 248 analyzer also calculates bicarbonate concentration within a range of 0.0 to 60.0 mmol/L using the formula:

$$\text{HCO}_{3\text{act}} = 0.0307 * \text{PCO}_2 * 10^{(\text{pH} - 6.105)}.$$

Oxygen saturation is measured within a range of 1% to 100% and calculated using the formula:

$$s\text{O}_2 = \frac{N^4 - 15 * N^3 + 2045 * N^2 + 2000 * N}{N^4 - 15 * N^3 + 2400 * N^2 - 31.100 * N + (2.4 \cdot 10^6)} \cdot 100$$

In which $N = \text{PO}_2 \times 10^{(0.48(\text{pH} - 7.4) - 0.0013\text{BE})}$

Total CO_2 is calculated using the formula:

$$\text{TCO}_2 = \text{HCO}_{3\text{act}} + (0.0307 * \text{PCO}_2).$$

Lactate measurement

For determination of L-lactate concentration, the stored plasma was thawed and 0.05% methyl orange solution, buffered with 3 mol/L potassium hydrochloride, was added and the plasma filtered. The L-lactate concentration was measured photometrically using the Roche Cobas Mira S analyzer^j and specific L-lactate dehydrogenase and nicotinamide adenine dinucleotides.^k

Data analysis

The results were entered in a spreadsheet,^l analyzed using a statistical add-in program,^m and displayed graphically. All values were plotted in a histogram and outliers were identified and verified.

Within-run precision

For determination of within-run precision, venous blood collected into a syringe from a healthy calf immediately after birth underwent 15 consecutive analyses with the i-STAT and the Rapidlab 248 and mean, standard deviation (sd), and coefficient of variation (CV) were calculated for each variable.

Comparison of methods

For comparison of results from the i-Stat and the Rapidlab 248 reference method, linear regression analysis was calculated and Bland–Altman plots of the difference between the test method (i-STAT) and reference method (Rapidlab 248) against the mean of the two methods were generated for each variable.¹⁵ Because the data set consisted of repeated and thus dependent measures, 95% limits of agreement (mean difference \pm 2 sd) were calculated according to Bland and Altman (2007) and Carstensen et al. (2008) using the statistical program Stata (StataCorp, College Station, Tx, USA)^{16,17} Pearson's coefficient of correlation (r), bias (mean of the difference), intercept, and slope with 95% confidence intervals were also calculated. Correlations with $r \geq 0.9$, between 0.7 and 0.9, between 0.7 and 0.5 and <0.5 were defined as very strong, strong, moderate, and weak, respectively. A P-value < 0.0001 was considered significant.

Agreement and clinical relevance

To determine agreement of the analyzers the inherent imprecision of both methods (i_c) were calculated using the formula $i_c = \sqrt{CV^2_{i-STAT} + CV^2_{Rapidlab}}$.¹⁸ The difference between the i-Stat and Rapidlab 248 is then expected to be within the interval of $0 \pm 1.96 * CV * \text{mean}$ in 95% of the measurements. To minimize the effect of systematic differences and to transfer the reference intervals (mean \pm 2sd) for newborn calves obtained from the Rapidlab 248 in a previous study to the i-STAT the differences of the mean were used

to calculate the values of the i-STAT.^{19,20} For variables with a distinct systematic proportional error (PO₂ and lactate) slopes and intercepts of the regression equation were used to adjust the results of the reference methods.

Additionally clinical acceptable limits were defined using the sd of the variables measured with the Rapidlab 248 in healthy newborn calves in the above mentioned study (pH \pm 0.13, PCO₂ \pm 12.5 mmHg, PO₂ \pm 5.8 mmHg, HCO₃ \pm 4.0 mmol/L, BE(ecf) \pm 6.0 mmol/L, sO₂ \pm 9.2 %, L-lactate \pm 2.46 mmol/L).¹⁹

To determine the clinical relevance of i-STAT measurements, reference ranges (mean \pm 2sd) for newborn calves obtained from the Rapidlab 248 were used for comparison.¹⁹ Values obtained by the i-STAT and the reference methods that were outside the reference range were identified and compared to determine whether data of the reference methods outside the reference range were accompanied by corresponding i-STAT measurements outside the reference range.

Results

Within-run precision

Means, standard deviations, and CVs of the 15 serial i-STAT measurements of all variables are shown in Table 1. BE had by far the largest variation and the pH the smallest.

Comparison between i-STAT and Rapidlab 248

Results of comparison between the i-STAT and the Rapidlab 248 reference method are shown in Table 2 and Figures 1 and 2. All variables had very strong correlations except PO₂, which had a strong correlation (r=0.817). L-lactate concentration and pH had the strongest correlations; however, L-lactate had a positive bias because of a proportional systematic error. Furthermore, all i-STAT readings for L-lactate of 20 mmol/L were excluded from regression analysis because the i-STAT analyzer displayed this reading for

concentrations greater than 20 mmol/L. This occurred in 15 samples, 8 of which also yielded a result >20 mmol/L using the photometric reference method. The photometrically measured L-lactate concentration was <20 mmol/L in the remaining 7 samples. When values that exceeded 20 mmol/L were included in the calculations, the resulting correlation coefficient ($r=0.984$) and bias (-0.98) were very similar to those shown in Table 2. In 6 of all samples, the i-STAT failed to generate a result for L-lactate.

There was only a minor systematic error for PCO_2 , whereas for PO_2 there was a systematic error causing the i-STAT to overestimate values below 18.71 mmHg and to underestimate values above 18.71 mmHg.

The calculated values of BE, HCO_3 and TCO_2 had a constant systematic error, and across the entire range the i-STAT values were on average 2.3 mmol/L, 1.9 mmol/L and 2.0 mmol/L lower than the values from the reference method, respectively. The regression scatterplot for sO_2 revealed a proportional systematic error, and in the Bland-Altman difference plot, sO_2 had the largest bias of all variables.

Agreement and clinical relevance

The inherent imprecisions of both methods are shown in Table 3. With exception of the BE, less than 95% of the measured values were inside the acceptable limits based on the combined inherent imprecision of the two analyzers. Due to systematic error between the measurements of the i-STAT and the reference methods the values of the i-STAT were adjusted using the differences of mean. 95% of the measurements of the PCO_2 and BE were within the limits. The measurements of the variable PO_2 , HCO_3 , and sO_2 were within the limits in 76 to 91% of the cases.

The defined limits of the analytical errors that are allowable without compromising the clinical interpretation of the values were met in more than 95% of all measurements with exception of pH and sO_2 . The difference between the measurements of the pH was within the limits in 94.1% and of the sO_2 within 80.9% of the cases (Table 3).

It is critical for clinical assessment of a patient that values exceeding the reference range be correctly identified. Several values that exceeded the reference range were not correctly identified by the i-STAT as being above the reference range (Table 4). This was exemplified by pO₂ and sO₂, for which 2 of 8 and 9 of 23 values above the reference range, respectively, were not recognized by the i-STAT. On the other hand, i-STAT generated 16 L-lactate results that spuriously exceeded the reference range and would have led to different clinical interpretations.

Discussion

Point-of-care-testing is commonplace in veterinary clinics but less common and more complicated in the field. Transportation of patient samples to a laboratory is costly and time consuming and causes a delay in specific treatment. This is particularly disadvantageous in farm animal neonatology because emergencies associated with dystocia are common. Affected calves often have marked hypoxia associated with mixed respiratory-metabolic acidosis, referred to as asphyxia. This condition can be life-threatening. Formulating a prognosis is feasible clinically but accurate assessment of the degree of acidosis is not possible.^{7,21} Measuring pH accurately is critical because this is used to calculate the amount of buffer solution needed to treat the neonate, to shorten the period of acidosis and thus to alleviate its deleterious effects.²² Furthermore, the clinician must have confidence that the measurements from a point-of-care blood gas analyzer are correct. The i-STAT has been used for blood gas and acid-base measurements in newborn calves²³ and has been validated for use in adult cattle.¹² This analyzer has not been validated for acidemic newborn calves, which commonly have massive derangements in blood gas and acid-base variables to a degree that is rare in adult cattle.

Within-run precision of the i-STAT was high for all variables except BE, which had a CV of 82.2%. This may have been because the i-STAT only reports BE as integers. Most measurements were between -10 and 10 and it is therefore possible that rounding the results to the nearest one caused large differences between individual serial measurements. The same effect could possibly have caused the relatively large CV of sO_2 (12%). Because values for sO_2 were larger than BE by one order of magnitude, the effect of rounding was less pronounced.

Of the variables that are directly measured by the i-STAT, pH and PCO_2 had excellent correlation with reference methods and high correlation coefficients of > 0.95 . Likewise, the Bland-Altman scatterplots showed small differences with a bias of 0.02 and 1.95, respectively, which are unlikely to affect clinical assessment significantly. However, 6 of 16 (37.5%) PCO_2 values that exceeded the upper reference limit measured with the reference analyzer were within the reference limit when measured with the i-Stat. In all of these 6 samples, the PCO_2 values measured with the reference analyzer exceeded the upper reference limit by less than 3 mmHg, and it is therefore possible that the small proportional systematic error was sufficient to generate an i-STAT value below the limit.

Similarly, 6 of 8 PO_2 values that were within the reference values measured with the i-STAT exceeded the upper reference limit measured with the reference analyzer, but this error has little clinical significance. The correlation coefficient calculated for PO_2 was the smallest of all variables and the i-STAT overestimated values in the lower pressure range and underestimated values in the higher pressure range. Distinct outliers were more common in PO_2 than in other variables. All but one outlier above 30 mmHg had considerably lower i-STAT readings than Rapidlab readings and this could have accounted for a shift in the regression line. PO_2 also had the smallest correlation coefficient ($r=0.86$) and largest variation of the variables examined in a study validating the i-STAT for adult cattle using a comparable reference method.¹² In the same study, pH and PCO_2 had correlations with the reference method (both $r = 0.98$) similar to the present study.

The best correlation between the i-STAT and reference method in this study occurred in L-lactate ($r = 0.984$) despite a proportional systematic error. As shown by the regression scatterplot, the difference between the two methods became greater as the concentrations increased and the higher values generated by the i-STAT shifted the lower limit of agreement to the negative range in the Bland-Altman plot. The systematic error did not generally interfere with the recognition of values above the upper reference limit by the i-STAT, but it did result in frequent i-STAT values that exceeded the upper reference limit. This was because the difference between the two methods was already 1.06 mmol/L at 5.38 mmol/L, which was the upper reference limit. L-lactate concentrations of up to 20 mmol/L and higher commonly occur in neonatal calves but are rare in mature cattle except in severely ill patients with a poor prognosis.²⁴ An L-lactate concentration that exceeds the upper reference limit normally indicates chronic tissue hypoxia and a guarded prognosis, and therefore a spuriously high L-lactate concentration would lead to an unfavorable clinical conclusion.

Correlation coefficients calculated in the present study agreed well with those from another study in mature cattle (BE 0.972 vs. 0.96, HCO_3^- 0.965 vs. 0.91, sO_2 0.91 vs. 0.89, TCO_2 0.981 vs. 0.95) that used a different reference method and different cartridges.¹² One reason for the relatively low correlation coefficient for sO_2 could be the relatively low correlation coefficient for PO_2 because the latter is used for the calculation of the former. The proportional systematic error that led to underestimation of values by the i-STAT with increasing sO_2 saturation could be another factor. The difference between the two measuring methods is reflected by the bias, which was the largest of all variables, although this could also have been caused, at least in part, by the different formulae used for calculation of sO_2 . The Bland-Altman scatterplot for sO_2 revealed a large variation of the differences and wide limits of agreement, which are compatible with a random error in addition to a systematic error. The random error could be related to the different calculations used for sO_2 because HCO_3^- concentration and BE are used in the i-STAT and Rapidlab 248, respectively, both of which had larger variations of the differences than the other variables. The reasons for this could not be determined in our study.

Although correlation between measurements of the i-STAT and reference method was generally good or excellent, differences identified between the two methods underline the need for validation of new blood analyzers for different patient populations and different clinical problems. These differences between the measurements of the i-STAT and the reference method in the acceptance limits based on the inherent imprecision of both methods were evident in all but one variable (BE), in which the limits in BE were unacceptably wide. This disagreement between the two methods was particularly evident in the variables pH and L-lactate. Therefore the results of the i-STAT and the reference methods were not interchangeable in all variables. Since systematic differences were observable the differences of the means were used for adjusting the values measured by the i-STAT. Less than 12% of the values were thus outside the limits in all variables except L-lactate. Hence, the results of i-STAT should be used with caution in a research study. Even the adjustment of the values obtained from the i-STAT did not lead to an agreement, which is required for a study or the establishment of reference intervals.²⁰ However, in a clinical setting differences calculated by using the imprecision of the analyzers are of minor clinical importance. A small disagreement such as in the pH of ± 0.02 can be considered clinically irrelevant. Differences larger than the sd of the measurements in healthy newborn calves could compromise a correct interpretation. For example, the bias of 2.3 mmol/L for BE could potentially lead to incorrect clinical interpretations. As a general rule, treatment of asphyxic neonatal calves is recommended when the pH is < 7.2 and BE < -5 mmol/L;²⁵ however, due to the bias for BE a number of measurements from the i-STAT yielded false low levels. The 15 calves that were treated based on pH and BE values from the reference method would also have been treated based on i-STAT results, but the hand-held analyzer would also have led to the treatment of 12 other calves. Most of the values of these calves just barely undercut the lower reference limit. It is therefore possible that small systematic errors and using 37°C as default temperature of analysis could generate an i-STAT value below the limit, because the reference range used in this study was established from temperature corrected values. However, it is unlikely that treating these calves would have a detrimental effect, because they also suffering from a metabolic acidosis. Values exceeding the upper reference limit were recognized less reliably but increases in these variables are clinically

irrelevant in newborn calves. The i-STAT is useful for the on farm measurement of blood gas and acid base parameters other than sO₂ in neonatal calves.. This device allows assessment of the severity of acidemia and administration of specific treatment. High L-lactate concentrations should be interpreted with caution because at higher levels agreement with the reference method was not optimal.

Footnotes

^a i-STAT, Axonlab, Baden, Switzerland.

^b Terumo Neolus, Terumo Europe N.V., Leuven, Belgium.

^c Vygonüle T, Vygon, Aachen, Germany.

^d Supramid, 3.5 metric, Braun-Melsungen, Melsungen, Germany.

^e Monovette, Sarstedt, Nümbrecht-Rommelsdorf, Germany.

^f NaCl 0,9% Fresenius, Fresenius Kabi, Bad Homburg, Germany.

^g Heparin „Bichsel“ 5000 IU/mL, Bichsel, Interlaken, Switzerland.

^h Greiner Bio one, Kremsmünster, Austria.

ⁱ Rapidlab 248 blood gas analyzer, Siemens, Erlangen, Germany.

^j Cobas Mira S analyzer Roche, Basel, Switzerland.

^k Enzytec L-Lactat Acid, Biopharm, Darmstadt, Germany.

^l Excel, Microsoft, Wallisellen, Switzerland.

^m StatEL, ad Science, Paris, France.

References

1. Mee JF, Berry DP, Cromie AR. Prevalence of, and risk factors associated with, perinatal calf mortality in pasture-based Holstein-Friesian cows. *Animal* 2008;**2**:613-620.
2. Mee JF. Newborn dairy calf management. *Vet Clin N Am-Food A* 2008;**24**:1-17.
3. Bleul U, Lejeune B, Schwantag S, et al. Blood gas and acid-base analysis of arterial blood in newborn calves. *Vet Rec* 2007;**161**:688-691.
4. Szenci O. Role of acid-base disturbances in perinatal mortality of calves. *Acta Vet Hung.* 1985;**33**:205-220.
5. Mülling M. Asphyxie des neugeborenen Kalbes. *Prakt Tierarzt Collegium Veterinarium.* 1976;**58**:78-80.
6. Schuijt G, Taverne MA. The interval between birth and sternal recumbency as an objective measure of the vitality of newborn calves. *Vet Rec.* 1994;**135**:111-115.
7. Szenci O. Correlations between muscle tone and acid-base balance in newborn calves: experimental substantiation of a simple new score system proposed for neonatal status diagnosis. *Acta Vet Hung.* 1982;**30**:79-84.
8. Erickson KA, Wilding P. Evaluation of a novel point-of-care system, the i-STAT portable clinical analyzer. *Clin. Chem.* 1993;**39**:283-287.
9. McCain SL, Flatland B, Schumacher JP, et al. Comparison of chemistry analytes between 2 portable, commercially available analyzers and a conventional laboratory analyzer in reptiles. *Vet Clin Path.* 2010;**39**:474-479.
10. Steinmetz HW, Vogt R, Kastner S, et al. Evaluation of the i-STAT portable clinical analyzer in chickens (*Gallus gallus*). *J Vet Diagn Invest.* 2007;**19**:382-388.
11. Verwaerde P, Malet C, Lagente M, et al. The accuracy of the i-STAT portable analyser for measuring blood gases and pH in whole-blood samples from dogs. *Res Vet Sci.* 2002;**73**:71-75.

12. Peiro JR, Borges AS, Goncalves RC, et al. Evaluation of a portable clinical analyzer for the determination of blood gas partial pressures, electrolyte concentrations, and hematocrit in venous blood samples collected from cattle, horses, and sheep. *Am J Vet Res.* 2010;**71**:515-521.
13. Szenci O, Taverne MAM, Takács E. A review of 126 Caesarean sections by blood gas and the acid-base status of newborn calves. *Theriogenology* 1989;**32**:667-673.
14. N. N. Abbott Diagnostics Manual, 2008.
15. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**1**:307-310.
16. Bland JM, Altman DG. Agreement between methods of measurement with multiple observations per individual. *J Biopharm Stat.* 2007;**17**:571-582.
17. Carstensen B, Simpson J, Gurrin LC. Statistical models for assessing agreement in method comparison studies with replicate measurements. *Int J Biostat.* 2008;**4**: 16.
18. Jensen AL, Kjelgaard-Hansen M. Method comparison in the clinical laboratory. *Vet Clin Pathol* 2006;**35**:276-286.
19. Bleul U, Schwantag S, Kahn W. Blood gas analysis of bovine fetal capillary blood during stage II labor. *Theriogenology* 2008;**69**:245-251.
20. Friedrichs KR, Harr KE, Freeman KP, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol* 2012;**41**:441-453.
21. Götz E. Einfluss der Geburt auf die Blutlaktatkonzentration beim neugeborenen Kalb – gleichzeitig eine Evaluation des portablen Blutgasanalysesystems i-STAT. Department of Farm Animals: Vetsuisse-Faculty University Zurich, 2012.
22. Bleul U, Bachofner C, Stocker H, et al. Comparison of sodium bicarbonate and carbicarb for the treatment of metabolic acidosis in newborn calves. *Vet Rec.* 2005;**156**:202-206.
23. Probo M, Giordano A, Moretti P, et al. Mode of delivery is associated with different hematological profiles in the newborn calf. *Theriogenology* 2012;**77**:865-872.

24. Coghe J, Uystepuyst CH, Bureau F, et al. Validation and Prognostic Value of Plasma Lactate Measurement in Bovine Respiratory Disease. *Vet J.* 2000;**160**:139-146.

25. Szenci O. Can we decrease the incidence of perinatal mortality in the cow? Animal health, animal welfare and biosecurity. Proceedings of 13th International Congress in Animal Hygiene, Tartu, Estonia 2007;407-411.

Figure 1: Regression scatterplots (left) and Bland-Altman plots of the difference (right). In the regression scatterplots the red line is the line of identity ($y = x$) and the black line is the line of best fit. In the Bland-Altman plots, the y-axis is the difference (reference method (Rapidlabs 248) - test method (i-STAT) and the x-axis is the mean of the two methods. The red line shows the bias (mean difference between methods). The green lines mark the 95% confidence interval (CI 95%). The blue lines are the 95% limits of agreement.

Figure 2: Regression scatterplots (left) and Bland-Altman plots of the difference (right). In the regression scatterplots the red line is the line of identity ($y = x$) and the black line is the line of best fit. In the Bland-Altman plots, the y-axis is the difference (reference method (Rapidlabs 248) - test method (i-STAT) and the x-axis is the mean of the two methods. The red line shows the bias (mean difference between methods). The green lines mark the 95% confidence interval (CI 95%). The blue lines are the 95% limits of agreement.

Table 1: Means, standard deviations, and coefficients of variation of serial i-STAT measurements of variables used to determine with-run precision of the i-STAT.

Table 1

Variable	Mean	Standard deviation	Coefficient of variation (%)
pH	7.30	± 0.01	0.1
pCO ₂ (mmHg)	57.90	± 1.86	3.2
pO ₂ (mmHg)	22.10	± 1.19	5.4
BE(ecf) (mmol/L)	-0.90	± 0.74	-82.2
HCO ₃ ⁻ (mmol/L)	26.10	± 0.57	2.2
sO ₂ (%)	29.30	± 3.52	12.0
TCO ₂ (mmol/L)	27.70	± 0.59	2.1
L-lactate (mmol/L)	9.10	± 0.17	1.9

Table 2: Correlation, linear regression with intercept and slope, bias and 95% limits of agreement of the Bland-Altman analysis between i-STAT and Rapidlab 248 reference method. Except the intercept of the regression model of PCO₂ and slope of sO₂ all intercepts and slopes were significantly different from 0 and 1, respectively.

Variable	Correlation coefficient (r)	Intercept with 95% CI	Slope with 95% CI	Bias with 95% CI	95% limits of agreement
pH	0.986	-0.31 (-0.48 to -0.14)	1.04 (1.02 to 1.06)	0.02 (0.02 to 0.03)	-0.02 to 0.07
pCO ₂ (mmHg)	0.978	0.41 (-1.22 to 2.03)	0.96 (0.93 to 0.99)	1.95 (1.68 to 2.21)	-2.93 to 6.81
pO ₂ (mmHg)	0.817	4.49 (2.59 to 6.38)	0.76 (0.68 to 0.83)	1.82 (1.35 to 2.3)	-5.86 to 9.30
BE (mmol/L)	0.972	-2.32 (-2.58 to -2.01)	1.01 (0.97 to 1.04)	2.3 (2.12 to 2.49)	-2.79 to 7.28
HCO ₃ ⁻ (mmol/L)	0.965	-1.73 (-2.83 to -0.64)	0.99 (0.96 to 1.03)	1.91 (1.75 to 2.07)	-0.72 to 4.56
sO ₂ (%)	0.91	0.61 (-1.67 to 2.9)	0.85 (0.8 to 0.9)	5.62 (4.85 to 6.39)	-7.39 to 18.35
TCO ₂ (mmol/L)	0.981	-1.84 (-3.05 to -0.62)	0.99 (0.96 to 1.03)	2.0 (1.84 to 2.17)	-1.27 to 5.26
L-lactate (mmol/L)	0.985	0.27 (0.07 to 0.46)	1.15 (1.12 to 1.18)	-1.0 (-1.14 to -0.86)	-3.24 to 1.18

Table 3: Acceptance limits based on the inherent imprecision of both analyzers ($i_c = \sqrt{CV^2_{i-STAT} + CV^2_{Rapidlab}}$) as well as clinical limits and percentage of measurement within the limits between i-STAT and Rapidlab 248 reference method.

	Acceptance limits		Corrected i-STAT values ^a		Clinical limits ^b	
Variable	Limits	Percentage within the limits	Percentage within the limits	± SD of 25 healthy newborn calves	Percentage within the limits	
pH	± 0.02	26.2	63.6	± 0.13	94.1	
pCO ₂ (mmHg)	± 4.22	87.5	94.9	± 12.5	99.6	
pO ₂ (mmHg)	± 3.95	80.5	76.4	± 5.8	96.3	
BE (mmol/L)	± 11.05	99.6	99.6	± 6.0	96.7	
HCO ₃ ⁻ (mmol/L)	± 2.01	55.9	88.2	± 4.0	97.8	
sO ₂ (%)	± 9.92	80.8	91.2	± 9.2 %	80.9	
L-Lactate (mmol/L)	± 0.26	23.3	39.7	± 2.46	97.8	

^a i-STAT values were corrected using the bias in case of a constant systematic error (pH, pCO₂, BE, HCO₃⁻, sO₂) or using intercepts and slopes of the regression equation in case of a proportional error (pO₂, lactate).

^b Clinical limits determined previously at the Clinic of Reproductive Medicine using the same reference methods.¹⁹ No clinical limits for TCO₂ was available for newborn calves.

418 Table 4: Number of correct and incorrect i-STAT measurements of variables outside the reference range.

Variable	Correctly Recognized		Not Correctly Recognized			
	< Lower Reference Limit	> Upper Reference Limit	Spuriously Lower Limit	< Reference	Spuriously Upper Limit	> Reference ranges*
pH	14/14	30/41	1/226		3/226	7.11 – 7.43
pCO ₂ (mmHg)	3/3	10/16	4/252		0/252	41 – 79.4
pO ₂ (mmHg)	2/3	2/8	1/260		0/260	9.5 – 39.1
BE (mmol/L)	9/9	41/73	1/189		2/189	-10.3 – 7.7
HCO ₃ ⁻ (mmol/L)	8/9	26/50	2/212		2/212	18-7 – 33.9
sO ₂ (%)	1/2	9/23	2/246		1/246	0.9 – 63.5
L-Lactate (mmol/L)	0/0	46/48	0/207		16/207	0.46 – 5.38

419 *Reference ranges determined previously at the Clinic of Reproductive Medicine using the same reference
 420 methods.^{15,16} No reference range for TCO₂ was available for asphyxic calves.